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Original Article

Dynamics and diversity of cultivable rhizospheric and endophytic bacteria during the growth stages of cilembu sweet potato (*Ipomoea batatas* L. var. *cilembu*)

Agustina Monalisa Tangapo,[a, 1] Dea Indriani Astuti,[b, 1] Pingkan Aditiawati[b, *]

[a] *Microbial Biotechnology Research Group, Bandung Institute of Technology, Bandung, Indonesia*
[b] *Department of Biology, Faculty of Mathematic and Natural Sciences, Sam Ratulangi University, Manado, Indonesia*

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ABSTRACT

Cultivable rhizospheric and endophytic bacteria were isolated from cilembu sweet potato during the 5 mth period post planting to assess the diversity and dynamics of its bacterial community. The number of colony forming units of rhizospheric bacteria was significantly higher than for the endophytic bacteria. The diversity and genera richness of the bacteria associated with cilembu sweet potato in the early stages of growth were higher than in the last stages. The different cultivable bacteria were identified using 16S rRNA gene sequencing as: Alphaproteobacteria (*Methylobacterium, Sphingomonas, Paracoccus*), Gammaproteobacteria (*Klebsiella, Enterobacter, Pseudomonas, Serratia*), Bacteroidetes (*Chryseobacterium, Sphingobacterium*), Firmicutes (*Exiguobacterium, Bacillus, Staphylococcus*) and Actinobacteria (*Streptomyces, Arthrobacter, Kocuria, Microbacterium, Micrococcus*). The nitrogen content in the soil may significantly affect the change of bacterial diversity in the rhizosphere during the growth of cilembu sweet potato. All isolates were capable of producing plant growth-promoting traits, alone or in combination, such as indole acetic acid production, phosphate solubilization, ammonia production, nitrogen fixation, cellulolytic and amylolytic activity.

Introduction

Sweet potato (*Ipomoea batatas* L. Lam) has been cultivated in many countries around the world and is used as staple food, as well as a raw material for animal feed and alcohol production, so that it ranks sixth as the most important food crop after rice, wheat, potatoes, maize and cassava (Food and Agriculture Organization, 2002). One of the most popular sweet potato varieties in Indonesia is cilembu sweet potato (*I. batatas* L. var. *cilembu*) from Cilembu village, West Java province. Cilembu sweet potato, also known as "Ubi Madu Cilembu" (cilembu honey sweet potato), has superiority in terms of taste, as it is sweeter than other sweet potatoes (Arifin, 2002; Solihin et al., 2016). The high demand for cilembu sweet potato as an export has pushed the increase in its production, which has become difficult due to land space limitations (Solihin et al., 2017). Several studies have shown the trademark cilembu sweet potato flavor and sweetness are hardly produced outside Cilembu village (Arifin, 2002; Solihin et al., 2018). Consequently, enhancing the growth of this commodity in other areas with similar environmental conditions has become an important focus of research. Cilembu soil with its physico-chemical characteristics, organic and inorganic nutrients provides a unique habitat for bacteria (Subroto, 2010; Nedunchezhiyan et al., 2012). Understanding plant-associated bacteria is very important because they play a crucial ecological role in nutrients recycling and plant growth promotion (Berg and Smalla, 2009). Marasco et al. (2013) reported the plant growth-promoting (PGP) potential of grapevine-associated bacteria under three different agroclimatic conditions and the majority of isolates showed multiple PGP activities, which promoted plant growth directly, indirectly or synergistically.

The association between bacteria and roots is in the soil, rhizosphere and endorhiza. The rhizosphere is defined as the zone of soil that is affected by exudates produced by living plant roots, while the soil habitat is not directly influenced by exudates supplied by plant roots (Mahaffee and Kloepper, 1997). Endophytes are defined as "all microorganisms which for all or part of their lifetime colonize internal plant tissues" (Hardoim et al., 2015). Some rhizospheric bacteria can penetrate and colonize within root tissues as endophytic bacteria. These bacteria have the potential to maintain the biotic diversity in plant-associated bio-communities (Lodewycks et al., 2002; Hardoim et al., 2015).

* Corresponding author.
E-mail address: pingkan@sith.itb.ac.id (P. Aditiawati).
[1] Equal contribution.

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The study of the role of bacterial diversity in species variety and abundance is important for sustainable development (Singh and Varaprasad, 2008). However, there is no information on the dynamics and diversity of the rhizospheric and endophytic bacteria of cilembu sweet potato or the functions of these bacteria. Although the number of studies on the productivity of cilembu sweet potato has increased, it is not easy to collect information about the bacterial communities associated with cilembu sweet potato. The objective of the current study was to isolate and characterize the bacteria in the rhizosphere and inside the roots (endophyte) of cilembu sweet potato during its cultivation process, focusing on the cultivable bacteria isolates and analyzing their abundance and diversity. The study also aimed to analyze the relation between bacterial diversity and soil parameters, as well as examining the functional properties of the bacteria. All this information will be beneficial in identifying the role of bacteria in the quality establishment of cilembu sweet potato.

Materials and methods

Sampling of plants and rhizosphere soil and isolation of bacteria

Plant and rhizosphere soil samples were collected from a sweet potato var. cilembu cultivation site at Cilembu (geographical coordinates: 6°54'24.5″ S, 107°50'46.3″ E), Pamulihan, Sumedang, West Java, Indonesia. The rhizospheric soil sampling was carried out each month for 5 mth after planting (R1-R5). At each sampling time, five plants were randomly harvested and the soil loosely adhering to the roots was shaken, the resulting roots were pooled and then, the rhizosphere soil was collected by brushing off the roots or tuberous roots per plant followed by homogenization by sieving (Marquez et al., 2014). In addition, a soil sample was taken before planting (S0). The sample of soil (10 g) was suspended in 90 mL of sterile phosphate-buffered saline and agitated at 150 revolutions per minute for about 30 min, then serially diluted and spread on nutrient agar (NA). The plates were incubated at room temperature for 24–72 hr in triplicate (Kumar et al., 2012; Marquez et al., 2014). The total numbers of cultivable bacteria were determined as colony forming units (CFUs) on nutrient agar plates using the dilution plate method with three replications each time. The cilembu sweet potato roots/tuberous roots were used for the isolation of endophytic bacteria (EG1-EG5). The roots were first surface sterilized to remove bacteria from the surface. The roots were washed with tap water and sterile distilled water followed by washing in 70% ethanol. The roots were then washed with sterile distilled water and incubated for 10 min with 5% NaOCl. To confirm that the sterilization process was successful, the samples were plated on NA and incubated for 24 hr at 28 °C. The samples without contamination were used for isolation of the endophytic bacteria. For isolation, the samples were homogenized under aseptic conditions, then serially diluted and spread on NA (Del Giudice et al., 2008; Kumar et al., 2012). The numbers of colonies obtained on all agar plates were counted. Individual bacterial colonies were purified by sub-culturing on fresh media and preserved on basal media containing glycerol at −20 °C.

Functional characterization of bacterial isolates

Plant growth-promoting activities were also screened, consisting of: IAA (indole acetic acid) production, phosphate (P) solubilization, ammonia productions and nitrogen fixation. The ability of isolates to produce IAA was evaluated in nutrient broth (NB) culture (Atlas, 2010), the colorimetric method of Gordon and Weber (1951) was used. The IAA production was observed as development of a pink-red color and the optical density was recorded at 530 nm. The mineral P-solubilizing ability of the isolates was determined on Pikovskaya's medium (Pikovskaya, 1984) amended with tricalcium phosphate as inorganic P. For ammonia production, bacterial isolates were grown in peptone water (peptone 5 g/L) and incubated for 48 hr at room temperature, and then 0.5 mL Nessler's reagent (Iwata et al., 2012) was added and development color indicated NH_3 production (Cappucino and Sherman, 1992). For nitrogen fixation, nitrogen free Malate media containing bromothymol blue as an indicator was used (Gothwal et al., 2008). The amylolytic activity was determined using the method of Hankin and Anagnostakis (1975) in NA medium containing 1% soluble starch and after incubation, 5 mL of 1% iodine solution was added to the plates; a clear zone around the colonies indicated amylase production. For cellulolytic activity, the bacterial colonies were grown on carboxymethylcellulose agar medium and incubated for 2 d. The plates were flooded with Congo red solution (1 mg/mL) for 15 min. The plates were destained using 1 M NaCl for 15 min and a clear zone of hydrolysis indicated cellulose degradation (Gupta et al., 2012).

Molecular identification

For polymerase chain reaction (PCR) amplification, a loopful from a bacterial colony was suspended in 30 μL of TE buffer (100 mM Tris HCl, 10 mM ethylenediaminetetraacetic acid, pH 8.0), and heated in a boiling water bath for 5 min. One μL of cell extract was used on a PCR template to amplify the 16S rRNA gene (Arturo et al., 1995) in a 50 μL reaction volume with Kapa Taq Extra HotStart ReadyMix kit (Kapa Biosystem; Wilmington, MA, USA). The universal bacterial primers 27F (5'- AGAGTTTGATC [A/C]TGGCTCAG-3') and 1492R (5'-G [C/T]TACCTTGTTACGACTT-3') were used in the reaction. PCR amplifications were performed using an T100 Thermal Cycler machine (Bio-Rad; Hercules, CA, USA) with the following program: 5 min for initial denaturation at 95 °C and then 35 cycles of 30 s min at 96 °C, 30 s at 65 °C, and 1 min at 72 °C and finally 10 min at 72 °C. The amplified genes were subjected to electrophoresis using 1.2% agarose gel with a size marker (1 kb DNA ladder).

The PCR products were sent to Macrogen Inc. (Seoul, Korea) for nearly full-length 16S rRNA (~1500 bp) sequencing. The basic local alignment tool (BLAST) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) was used as to find similar known sequences.

Statistical analysis

All calculation of diversity indices were performed using the computer program PAST (Paleontological Statistics), version 3.14 (Hammer et al., 2001). The PAST software was adopted to measure the Shannon-Wiener (H') (formula: $H' = \sum pi \ln pi$) where H' the diversity index and pi is the proportion of species i to the total number of species. Species richness (S) is the total number of different species in each sample. The evenness index (E) provides information about the distribution of individual over species (Heip et al., 1998).

All enumerations of total numbers of CFU were analyzed using Annova 5% and then followed with Tukey's Method. The correlation between the soil properties and microbial diversity was determined using principal component analysis (PCA) using the correlation matrix to calculate eigen values and eigenvectors (using the PAST program).

Results and discussion

Isolation of bacteria during the growth of cilembu sweet potato

The bacterial abundance of rhizospheric bacteria during the growth of cilembu sweet potato substantially increased during the first 2 mth of growth. The abundance peaked in the second month of the growth phase (R2) with 13.07×10^6 CFU/g (Fig. 1). After the

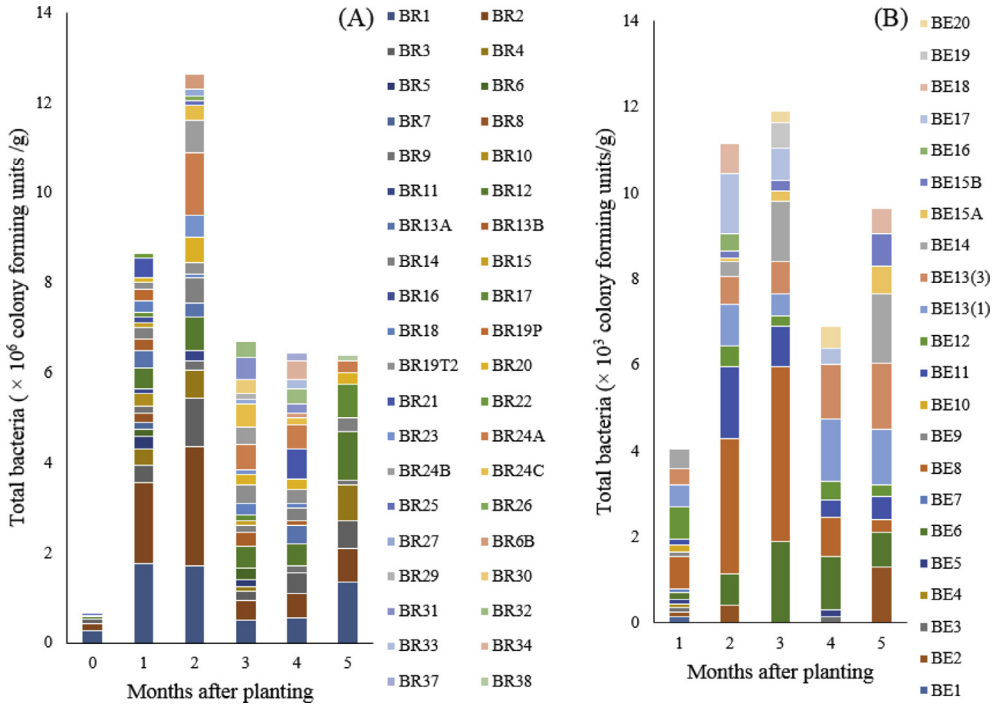

Fig. 1. Changes in total abundance during growth stages of cilembu sweet potato of: (A) rhizospheric bacteria isolates; (B) endophytic bacteria isolates.

second month, this value continued to fall to 6.60 × 10^6 CFU/g in the third month (R3) and then to 5.80 × 10^6 CFU/g in the fourth month (R4). The bacterial density and number of isolates of rhizospheric bacteria (R1-R5) were higher than in non-rhizospheric bacteria (soil before planting; S0). In total, 40 morphologically different bacteria were isolated from the rhizosphere of different-aged sweet potato variety cilembu. The highest species richness was in the first month of growth (24 of 40 isolates), while the lowest number was in the soil before planting (S0) with nine isolates. In the rhizosphere, the bacteria abundance in the early stages of growth was significantly higher than for the last stages. Plant growth and development stages influence the abundance and diversity of rhizospheric bacteria and it has been reported that the composition of root exudates changes according to plant growth and development stage (Chaparro et al., 2013). The concentrations of sugar and sugar alcohols in the root exudates were higher earlier on and decreased with plant growth, while amino acids and phenolics increased over time. Based on this information, it was assumed that in early of growth stages when roots were growing quickly, sugar was released as substrate for a diversity range of bacteria in their early stages of development (Badri et al., 2013; Chaparro et al., 2013).

In the endophytes, a significantly higher abundance was also observed in the mid-growth stages from the root/tuberous root samples compared to the last 2 mth. The total numbers of bacteria from the root/tuberous root of cilembu sweet potato were between 4.05 × 10^3 and 11.9 × 10^3 CFU/g of wet sample and reached the highest abundance in the third month of growth (Fig. 1). The decrease in the bacterial population in the last stages could have been due to the unavailability of essential nutrients for bacteria during storage root growth (bulking) stages of cilembu sweet potato. Changes in plant physiology can lead to the development of a distinct endophytic bacteria community (Hallman et al., 1997). The bacterial density of the rhizosphere was significantly greater than of endophytes for all sampling times. Endophytic bacteria are mostly derived from the rhizosphere as endophytic bacteria communities represent a subgroup of the rhizobacterial communities, which has the ability to enter the root interior of the plant and thus, there is a selection of the bacteria in the rhizosphere that colonize the plant (Sessitsch et al., 2002).

Diversity of bacteria during the growth of cilembu sweet potato

The diversity of isolates was calculated for each month based on several indices. The Shannon index (H') accounts for both the abundance and evenness, and may explain the diversity of bacteria (Stirling and Wilsey, 2001). H' analysis indicated that the diversity of the bacteria in the rhizosphere was highest in the third month sample (R3, $H' = 2.83$) and lowest in the fifth month sample (R5, $H' = 2.06$), whereas for the endophytic bacteria it was highest in the first month sample ($H' = 2.34$) and lowest in the samples in the third and fourth months (EG3 and EG4, $H' = 2.01$). These values were higher than the H′ value from the soil before planting (S0, $H' = 1.68$) as shown in Table 1. This result showed that there was a significant increase in bacterial diversity during the growth period of cilembu sweet potato, which peaked in the third month of growth for the rhizosphere and in the first month for endophytes. Moreover, the range in the evenness index for the rhizosphere was 0.85–0.98 and for the endophytes was 0.83–0.93, where a higher value reflects a uniform distribution of the individuals in each species (Table 1). There was no dominant species in any sample. However, several isolates, (BR1, BR2, BR3, BR12 for rhizospheric bacteria and BE6, BE8, BE11, BE12, BE13 (1), BE13 (3) for endophytic bacteria0 were always detected each sampling time (Fig. 1).

Significant changes in bacterial diversity were found during the growth of cilembu sweet potato perhaps as a result of many different factors such as plant species and cultivars, plant developmental stage, properties of soil and the season (Berg and Smalla, 2009). The presence of crops in the soil may change the soil properties by producing plant exudates, and cause a rapid increase in bacterial diversity and abundance in the rhizosphere as was seen in the first month. The release of root exudates may become a

Table 1
Diversity indices for bacterial communities from different ages of cilembu sweet potato.

Sample	Age (mth)	Species richness (*S*)	Shannon index (*H′*)	Evenness_index (*E*)
Soil before planting	0 (S0)	9	1.68	0.82
Rhizosphere	1 (R1)	24	2.61	0.85
	2 (R2)	20	2.49	0.87
	3 (R3)	23	2.83	0.97
	4 (R4)	20	2.72	0.98
	5 (R5)	11	2.06	0.92
Endophytes	1 (EG1)	15	2.34	0.87
	2 (EG2)	12	2.08	0.83
	3 (EG3)	12	2.01	0.86
	4 (EG4)	10	2.01	0.91
	5 (EG5)	11	2.20	0.93

source of carbon compounds for growth substrates or a signal for root-associated microorganisms (Compant et al., 2010). Plant development stages may also influence the variety and amount of exudates produced, which could serve as a selective pressure in the rhizosphere. This includes the bacteria, which have an important role during plant growth. Cilembu sweet potato is known to start forming tubers during the second month of its growth (Van de Fliert and Braun, 1999), which may change the amount and type of exudates produced. This finding may explain the change in bacterial diversity and abundance in the second month, when the species richness began to drop due to the selective pressure by the produced exudates. As the number of species decreased, the abundance of the remaining bacteria increased, as was seen in the third month of growth.

Soil characteristics: rhizospheric bacteria diversity relationship

The production of exudates by plants may cause some changes in the chemical and physical properties of the soil including the pH, total C, N, and available P and K contents (Nardi et al., 2000). These properties are known to affect the general composition of the bacterial community. Changes in bacterial diversity and dynamics in the rhizosphere during the development stages of plants are the result of differences in the physical, chemical and biological properties of the root-associated soil (Berg and Smalla, 2009). The PCA results showed the relationships between bacterial diversity (*H′*) and soil parameters (Fig. 2). The soil parameters used in this study have been reported by Nasution et al. (2017). Soil pH in the rhizosphere soil was in the range 4.8–5.4 and was lower than in bulk soil (5.9). The N values in the rhizosphere soil were in the range 0.16–0.29%.

Component 1 from the PCA explained 74.5% and component 2 12.3% of the total variation. The N content had a direct correlation with bacterial diversity (*H′*), while the C:N ratio had an inverse correlation (Fig. 2). These findings may suggest that the N content, as a chemical property of soil, may significantly affect the change in bacterial diversity in the rhizosphere during the growth of cilembu sweet potato. The lowest N content had the highest bacterial diversity (sample R1). Based on Bobbink et al. (2010), N accumulation can cause a decline in microbial diversity by the expansion of nitrophilous species and the competitive exclusion of others. The current study also recorded a decrease in the soil pH with an increase in N. The increase in N can induce soil acidification, exerting deleterious effects on microbial growth (Wei et al., 2013). Some studies suggested that soil texture and pH are parameters that might affect the richness and diversity of bacterial communities in the soil (Sessitsch et al., 2002; Beneduzzi et al., 2013).

Molecular identification of bacterial isolates

All isolates were characterized morphologically and identified using PCR amplification with the 16S rRNA gene (Table 2). The identified bacterial genera were: *Klebsiella, Enterobacter, Pseudomonas, Chryseobacterium, Sphingomonas, Micrococcus, Kocuria,*

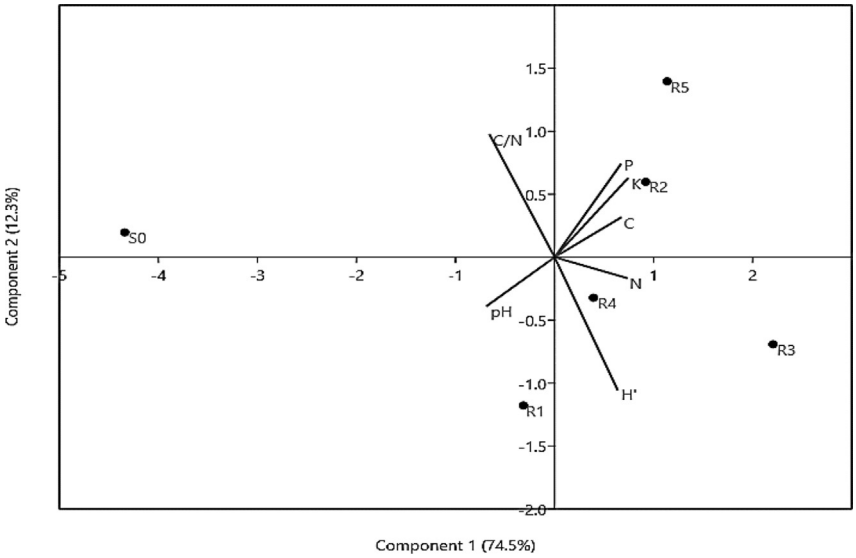

Fig. 2. Statistical correlation between soil composition (pH, C, N, P, K, C/N) and diversity index (*H′*) where nitrogen and *H′* are located in the same quadrant, which indicates a significant correlation.

Table 2
Identification of rhizospheric and endophytic bacteria of cilembu sweet potato using NCBI BLAST-N of 16S rRNA gene sequences.

Isolates	Closest match/species identity	Family	Phylum
Rhizospheric bacteria			
BR1	*Klebsiella pneumoniae* NR 041750 (99%)	Enterobacteriaceae	Proteobacteria
BR2	*Enterobacter cloacae* NR 118568 (99%)	Enterobacteriaceae	Proteobacteria
BR3	*Pseudomonas nitroreducens* NR 113601 (99%)	Pseudomonadaceae	Proteobacteria
BR4	*Chryseobacterium* indologenes NR112975 (98%)	Flavobacteriaceae	Bacteroidetes
BR5	*Sphingomonas paucimobilis* NR 114999 (99%)	Sphingomonadaceae	Proteobacteria
BR6	*Micrococcus luteus* NR 114673 (99%)	Micrococcaceae	Actinobacteria
BR7	*Kocuria rhizophila* NR 026452 (99%)	Micrococcaceae	Actinobacteria
BR8	*Sphingomonas endophytica* NR117869 (100%)	Sphingomonadaceae	Proteobacteria
BR9	*Microbacterium laevaniformans* NR 115540 (99%)	Microbacteriaceae	Actinobacteria
BR10	*Exiguobacterium acetylicum* NR 113585 (99%)	Bacillaceae	Firmicutes
BR11	*Sphingobacterium multivorum* NR 113076 (94%)	Sphingobacteriaceae	Bacteroidetes
BR12	*Bacillus mycoides* NR 036880 (99%)	Bacillaceae	Firmicutes
BR13A	*Bacillus mojavensis* NR 024693 (99%)	Bacillaceae	Firmicutes
BR13B	*Bacillus pumilus* NR 112637 (99%)	Bacillaceae	Firmicutes
BR14	*Bacillus licheniformis* NR 074923 (99%)	Bacillaceae	Firmicutes
BR15	*Streptomyces albus* NR 118467 (99%)	Streptomycetaceae	Actinobacteria
BR16	*Streptomyces sp.* NR 112305 (99%)	Streptomycetaceae	Actinobacteria
BR17	*Streptomyces abikoensis* NR 118286 (99%)	Streptomycetaceae	Actinobacteria
BR18	*Streptomyces griseus* NR 115669 (98%)	Streptomycetaceae	Actinobacteria
BR19P	*Methylobacterium platani* NR 044211 (99%)	Methylobacteriaceae	Proteobacteria
BR19T	*Bacillus aquimaris* NR 025241 (95%)	Bacillaceae	Firmicutes
BR20	*Bacillus simplex* NR 115603 (99%)	Bacillaceae	Firmicutes
BR21	*Bacillus megaterium* NR 117473 (97%)	Bacillaceae	Firmicutes
BR22	*Bacillus aryabhattai* NR 115953 (100%)	Bacillaceae	Firmicutes
BR23	*Bacillus badius* NR 112633 (98%)	Bacillaceae	Firmicutes
BR24A	*Bacillus subtilis* NR 116017 (100%)	Bacillaceae	Firmicutes
BR24B	*Bacillus cereus* NR 074540 (99%)	Bacillaceae	Firmicutes
BR24C	*Bacillus flexus* NR 113800 (94%)	Bacillaceae	Firmicutes
BR25	*Bacillus sp.* MF 948374 (99%)	Bacillaceae	Firmicutes
BR26	*Bacillus acidiceler* NR 043774 (99%)	Bacillaceae	Firmicutes
BR27	*Streptomyces sp.*S000651623 (80%)	Streptomycetaceae	Actinobacteria
BR6B	*Arthrobacter ureafaciens* NR 029281 (97%)	Micrococcaceae	Actinobacteria
BR29	*Serratia marcencens* NR 114043 (99%)	Enterobacteriaceae	Proteobacteria
BR30	*Paracoccus sp.* NR 149253 (97%)	Rhodobacteraceae	Proteobacteria
BR31	*Bacillus sp.* NR 113945 (95%)	Bacillaceae	Firmicutes
BR32	*Staphylococcus saprophyticus* NR 074999 (99%)	Staphylococcaceae	Firmicutes
BR33	*Unidentified*		
BR34	*Bacilllus amyloliquefaciens* NR 041455 (99%)	Bacillaceae	Firmicutes
BR37	*Bacillus methylotrophicus* NR 116240 (99%)	Bacillaceae	Firmicutes
BR38	*Bacillus luciferensis* NR 025511 (95%)	Bacillaceae	Firmicutes
Endophytic bacteria			
BE1	*Bacillus mycoides* NR 113990 (99%)	Bacillaceae	Firmicutes
BE2	*Klebsiella pneumoniae* NR 041750 (99%)	Enterobacteriaceae	Proteobacteria
BE3	*Unidentified*		
BE4	*Unidentified*		
BE5	*Arthrobacter ureafaciens* KT 380556 (99%)	Micrococcaceae	Actinobacteria
BE6	*Pantoea* NR 111998 (94%)	Enterobacteriaceae	Proteobacteria
BE7	*Streptomyces abikoensis*NR 118286 (99%)	Streptomycetaceae	Actinobacteria
BE8	*Enterobacter cloacae* NR 118568 (98%)	Enterobacteriaceae	Proteobacteria
BE9	*Microbacterium laevaniformans* NR 115540 (99%)	Microbacteriaceae	Actinobacteria
BE10	*Paracoccus communis* NR 133809 (97%)	Rhodobacteraceae	Proteobacteria
BE11	*Unidentified*		
BE12	*Bacillus subtilis* NR 113265 (99%)	Bacillaceae	Firmicutes
BE13-1	*Bacillus mojavensis* NR 112725 (99%)	Bacillaceae	Firmicutes
BE13-3	*Bacillus badius* NR 112633 (98%)	Bacillaceae	Firmicutes
BE14	*Bacillus megaterium* NR 117473 (99%)	Bacillaceae	Firmicutes
BE15A	*Bacillus licheniformis* NR 074923 (99%)	Bacillaceae	Firmicutes
BE15B	*Bacilllus amyloliquefaciens* NR 041455 (99%)	Bacillaceae	Firmicutes
BE16	*Bacillus safensis* NR 113945 (95%)	Bacillaceae	Firmicutes
BE17	*Sphingobacterium multivorum* NR 113706 (94%)	Sphingobacteriaceae	Bacteroidetes
BE18	*Staphylococcus haemolyticus* NR 113345 (100%)	Staphylococcaceae	Firmicutes
BE19	*Pseudomonas putida* NR 113651 (98%)	Pseudomonadaceae	Proteobacteria
BE20	*Bacillus pumilus* NR 112637 (99%)	Bacillaceae	Firmicutes

Microbacterium, Sphinghobacterium, Exiguobacterium, Bacillus, Streptomyces, Paracoccus, Staphylococcus, Methylobacterium, Arthrobacter and *Serratia*. The results showed that all the classified isolates of rhizospheric bacteria belonged to 12 families: the Bacillaceae (47.5%), Streptomycetaceae (12.5%), Enterobacteriaceae (7.5%), Micrococcaceae (7.5%), Sphingomonadaceae (5%), Pseudomonadaceae (2.5%), Flavobacteriaceae (2.5%), Microbacteriaceae (2.5%), Methylobacteriaceae (2.5%), Sphingobacteriaceae (2.5%), Rhodobacteraceae (2.5%) and Staphylococcaceae (2.5%). Furthermore, all the classified isolates of endophytic bacteria belonged to nine families: the Bacillaceae (40.9%), Enterobacteriaceae (13.6%), Micrococcaceae (4.5%), Streptomycetaceae (4.5%), Microbacteriaceae (4.5%), Rhodobacteraceae (4.5%), Sphingobacteriaceae (4.5%), Staphylococcaceae (4.5%) and Pseudomonadaceae (4.5%). All the isolates

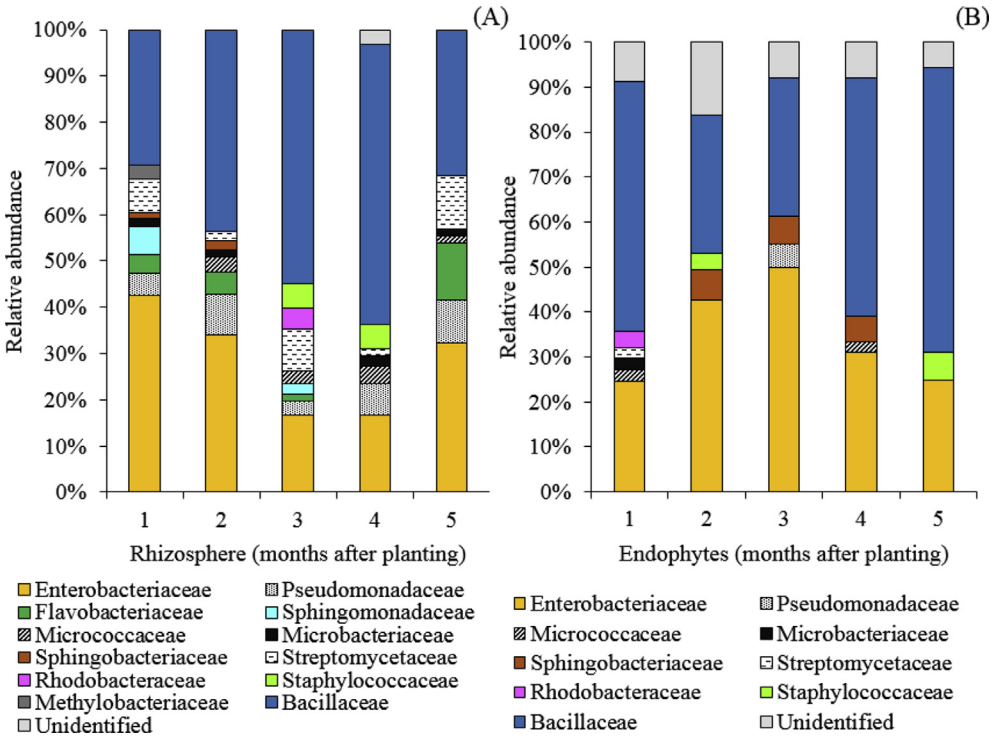

Fig. 3. Relative abundance each month during growth stages of: (A) rhizospheric bacteria; (B) endophytic bacteria.

could be attributed to four phyla (Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes), which have been known to be plant-associated (Reinhold-Hurek et al., 2015). Molecular identification using 16S rRNA gene sequencing showed *Bacillus* was the dominant genus of rhizospheric and endophytic bacteria. This was consistent with the study in sweet potato rhizosphere by Marquez et al. (2014), where the genus *Bacillus* was enriched in the tuber rhizosphere samples of all sweet potato genotypes studied, while other genera showed a plant genotype-dependent abundance.

Species richness and estimation of the diversity of rhizospheric bacteria provide information on their composition and roles in the environment. The present study found that the presence of the Enterobacteriaceae, Pseudomonadaceae, Bacillaceae and Streptomycetaceae was not influenced by plant age, as they were detected on all sampling occasions. In contrast, the Methylobacteriaceae were only present in the first month sample (R1) as shown in Fig. 3. The isolates BR1 and BR2 were the most abundant isolates in the majority of the rhizosphere soil samples, and were also found at all sampling times. The analysis showed that isolate BR1 had the highest sequence similarity to *Klebsiella pneumonia subsp. ozaenae*, while isolate BR2 was similar to *Enterobacter cloacae* (Table 2). A previous study showed that the *Klebsiella* strain was present in the rhizosphere and showed PGP traits (Sachdev et al., 2009). *Enterobacter* spp. were also found in the rhizosphere of sugarcane (Mirza et al., 2001). The presence of *Enterobacter* was also detected as endophytic bacteria (BE8).

Functional characterization of rhizospheric and endophytic bacteria

All the rhizospheric and endophytic bacteria of the cilembu sweet potato were further screened for the presence of PGP traits in order to understand their role in their habitat. The PGP traits evaluated in this study were: IAA production, phosphate solubilization, ammonia production, nitrogen fixation, cellulolytic and amylolytic activity. The results showed that more than 95% of the rhizospheric and endophytic bacteria possessed more than one PGP

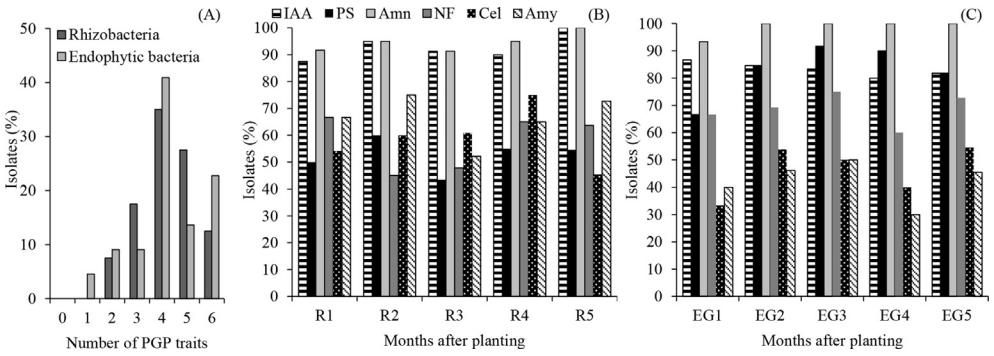

Fig. 4. Plant growth-promoting (PGP) potential of rhizospheric and endophytic bacteria of cilembu sweet potato: (A) percentage of isolates showing multiple PGP traits. Percentage of isolates showing PGP traits during growth stages for IAA production (IAA), phosphate solubilization (PS), ammonia production (Amn), nitrogen fixation (NF), cellulolytic (Cel) and amylolytic (Amy) activity of: (B) rhizospheric bacteria (R); (C) endophytic (EG) bacteria.

trait, which may promote plant growth synergistically. None of the rhizospheric bacteria and only 4.5% of the endophytic bacteria showed only one or no activity, while about 86% of isolates from both location showed more than two PGP traits (Fig. 4A). Only 12.5% (5 out of 40) of the rhizospheric bacteria were positive for IAA production, phosphate solubilization, ammonia production, nitrogen fixation and cellulolytic and amylolytic activity (six PGP traits) while 22.7% of the endophytic bacteria were positive for six PGP traits. Similar findings were reported by Marasco et al. (2013) who stated that the majority of grapevine-associated bacteria showed multiple PGP activities, with about 80% of isolates from the rhizosphere and endophyte fractions displaying more than three PGP traits. In another study, Susilowati et al. (2015) confirmed that 20.5% (16 of 78) isolates of rhizobacteria of the rice plant in the coastal soils of Indonesia had a positive result for three PGP traits (IAA production, nitrogen fixation, phosphate solubilization). Furthermore, bacterial isolates that had been obtained from the rhizosphere of sugarcane, corn, chili and watercress showed multiple PGP traits for siderophore production, phosphate-solubilizing ability and the production of auxin, gibberellin and acylhomoserine lactone (Dangjarean et al., 2015).

The percentage of isolates of IAA production from the rhizosphere (87.5–100%) was higher than for endophytes (80–86.7%) (Fig. 4B, C). IAA production is one of the most important factors for plant growth promotion by microorganisms (Etesami et al., 2015). It is assumed that the rhizospheric bacteria produce auxins in the plant rhizosphere upon the release of tryptophan in the root exudates that can influence the growth of plants (Asghar et al., 2002). About 80% of the rhizospheric bacterial in several crops displayed the ability to produce and release auxins as secondary metabolites (Pattern and Glick, 1996). In the rhizosphere, isolates that could produce IAA and ammonia (93.3–100%) were more abundant than for other PGP traits during growth stages. In the endophytic bacteria, ammonia production (93–100%) was higher than IAA production for the five months since planting (Fig. 4B, C). Ammonia production can influence plant growth promotion indirectly by supplying nitrogen to the plant. Moreover, phosphate solubilization ability was displayed by 43–60% of the rhizospheric bacteria and by 66–91% of the endophytic bacteria (Fig. 4B, C). a large amount of total phosphorus is available in the soil, but its availability is very low to plants and is important for plant growth (Pradhan and Sukla, 2006). In the current study, the rhizospheric bacteria showed cellulolytic (45–74%) and amylolytic (65–75%) activity during the five months post planting while endophytic bacteria showed 33–54% cellulolytic and 30–50% amylolytic activity (Fig. 4B, C). The cellulolytic and amylolytic activity of bacteria were important in organic matter decomposition and for plant growth promotion through the inhibition of soil pathogens.

In conclusion, this study described the cultivable diversity and functional activities of the rhizospheric and endophytic bacteria of cilembu sweet potato during its growth stages. By comparing the population of bacteria each month, the data implied that abundance (including the number of isolates, the number of CFUs and the diversity indices) in the early growth stages for rhizospheric bacteria and in the mid-growth stages for endophytic bacteria, were higher than in the late stages. This phenomenon might have been related to the change in secreted root exudates. The genus *Bacillus* was dominant in the rhizosphere and endophytes of cilembu sweet potato. Multiple PGP activities among rhizospheric and endophytic bacteria were found; for example, IAA production, phosphate solubilization, ammonia production and nitrogen fixation were exhibited by multiple strains in all stages of growth of cilembu sweet potato.

Conflict of interest

The authors declare that there are no conflicts of interest.

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